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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel benzimidazole–oxadiazole derivatives as potent anticancer activity

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Abstract

DNA topoisomerase I regulates DNA topological structure in many cellular metabolic processes and is a validated target for the development of antitumor agents. In this work, a series of novel 2-[(5-(4-(5(6)-substituted-1*H*-benzimidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-yl)thio]-1-(4-substitutedphenyl)ethan-1-ones (**4a–4s**) derivatives have been synthesized and evaluated for DNA Topo I inhibition and cytotoxicity. The structures of the compounds (**4a–4s**) were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, 2D NMR, and mass spectroscopy. Anticancer activity of these compounds was assessed against two different human cancer cell lines A549 (human lung adenocarcinoma) and HepG2 (human liver cancer cell line), as well as normal mouse embryonic fibroblast cells (NIH3T3). IC_{50} values of compounds **4a**, **4c**, and **4f** were highest than those exhibited for the reference drug cisplatin. Then, the inhibitory effect of **4a**, **4c**, and **4f** compounds on topoisomerase I enzyme with the relaxation assay was investigated on supercoiled DNA using agarose gel electrophoresis. The Annexin V-FITC assay demonstrated that these compounds induce cell death by apoptosis.

Keywords Benzimidazole · 1,3,4-Oxadiazole · Anticancer · DNA flow cytometric · 2D NMR

Introduction

Cancer, the main cause of universal death, is a major health risk, characterized by abnormal growth of cells that proliferate uncontrollably and metastasize to nearby organs through the bloodstream or lymph system (El-Goharya et al. 2019; Abou-Zied et al. 2019). To date, several cytotoxic drugs have been developed that are ready to fight cancer. On the other hand, the narrow therapeutic efficacy and undesirable effects of most of these drugs limit their using for clinically (Haider et al. 2019). Therefore, there is a need to develop new effective anticancer drugs with less side effects. As a great percentage of chemotherapeutic drugs presently used in cancer therapeutics are DNA-modifying and/or DNA-binding agents, such as topotecan, cisplatin, adriamycin, etc. (Mahanti et al. 2019). The antitumor activity is due to the intercalation between the base pairs of DNA and interferences with the normal functioning of enzyme topoisomerase, which is involved in the breaking and releasing of DNA strands. Topoisomerase 1 (Top1) functions by separating a strand of DNA by a transesterification reaction by the nucleophilic attack to the DNA phosphodiester backbone of catalytic tyrosine (Tyr723 for human Top1) to form enzyme–DNA covalent cloning complexes (Top1cc) (Tang et al. 2019). Topo I, which inhibitors are frequently used in the clinic, plays a critical role in cell proliferation and is considered an important target for the prevention rapid proliferation of cancer cells (Zhang et al. 2019). From the literature survey, it was evident Topoisomerase 1 (Top) expresses itself constitutively in severel forms of cancer (breast, leukemia,

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lymphoma, lung, prostate, and ovary) and has vital roles in cancer cell growth, survival (Haider et al. 2019).

The derivatives of benzimidazole have been widely used in different biological activity (Akkoç 2019) research are as like anticancer (El-Gohary and Shaaban (2017)), antitubercular (Ranjith et al. 2013), antiallergic (Caselli et al. 1988), antiinflammatory (Abraham et al. 2018), antiviral (Starčević et al. 2007), antibacterial (Mahmood et al. 2019), antifungal (Chandrika et al. 2016). In addition, benzimidazoles have been reported to be an anticancer agent (O'Donovan et al. 2008; Abdel-Mohsen et al. 2010; Refaat 2010; Demirayak et al. 2011; Lewis et al. 2012; Mavrova et al. (2013); Shi et al. 2014). Hoechst 333424 and Hoechst 33258 including benzimidazole ring are two equally potent topoisomerases I poison when tested using purified calf thymus DNA topoisomerase I (Chen et al. 1993). In addition, the oxazole, isoxazole, and oxadiazole ring an advantaged core in numerus antitumor molecules like SEW2871(EPICorporation), MX74420 (MaximPharmaceuticals), VA-62784. Remarkably, oxadiazole derivatives displayed promising antitumor activities with different modes of actions. The mentioned heterocyclic rings present promising scaffolds in the design of DNA-interacting agents (Mohammed 2019).

For these reasons, there has been a desire to develop a new topoisomerase inhibitor. Compounds containing benzimidazole and oxadiazole rings were synthesized. The reason for selecting these two ring systems is that there are studies showing that both rings show anticancer activity by DNA interaction. Therefore, this study was carried out to elucidate the effect of compounds containing these two rings on topoisomerase I enzyme.

Material and methods

Chemistry

Whole chemicals employed in the synthetic procedure were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points of the obtained compounds were determined by MP90 digital melting point apparatus (Mettler Toledo, OH, USA) and were uncorrected. The IR spectra were obtained on a Shimadzu, IR Prestige-21 (Shimadzu, Tokyo, Japan). ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized compounds were registered by a Bruker 300 and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet in the NMR spectra. Coupling constants (J) were reported as Hertz. M+1 peaks were determined by Shimadzu LC/MS ITTOF system (Shimadzu, Tokyo, Japan). All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany).

Synthesis of methyl 4-(5(6)-substituted-1H-benzimidazol-2-yl)benzoate (1a, 1b)

The compound (1a or 1b) was synthesized according to previously published procedure (Acar Cevik et al. 2017). 4-Formylbenzoic acid methyl ester (3.2 g, 0.02 mol) in DMF was reacted with sodium metabisulfite (3.8 g, 0.02 mol) at microwave synthesis reactor (Anton-Paar Monowave 300) for 5 min. After then, 5-substitute-1,2-phenylenediamine (0.02 mol) was added in the mixture and kept under the same reaction conditions. The mixture was poured into ice water to give the product.

Synthesis of 4-(5(6)-substituted-1H-benzimidazol-2-yl) benzohydrazide (2a, 2b)

The compound (2a or 2b) was synthesized according to previously published procedure (Acar Cevik et al. 2017). Methyl 4-(5(6)-substituted-1*H*-benzimidazol-2-yl)benzoate (1a, 1b) (0.02 mol) was dissolved in 20 mL ethanol, and 5 mL hydrazine hydrate was added to the solution. The reaction mixture was heated at 240 °C and 10 bar for 10 min at microwave synthesis reactor (Anton-Paar Monowave 300). The mixture was poured into ice water to give the product.

Synthesis of 5-[4-(5(6)-substituted-1H-benzimidazol-2-yl) phenyl]-1,3,4-oxadiazole-2-thiol derivatives (3a, 3b)

Compounds 2a or 2b (0.031 mol) were dissolved in a solution of NaOH (1.48 g, 0.037 mol) in ethanol (150 mL) and carbon disulfide (2.24 mL, 0.037 mol) was added in the mixture. The mixture was refluxed for 8 h. When the reaction was finished, the solution was cooled and acidified to pH 4–5 with concentrated hydrochloric acid solution to obtained compounds (3a, 3b).

Synthesis of 2-[(5-(4-(5(6)-substituted-1H-benzimidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-yl)thio]-1-(4-substitutedphenyl) ethan-1-ones (4a–4s)

Compounds 3a or 3b (2 mmol), 4-substituted phenacyl bromide (2 mmol), and potassium carbonate (0.33 g, 2.4 mmol) were mixed in acetone (40 mL) at room temperature for 8 h. After the reaction was ended, the product was washed with water, dried, and crystallized from ethanol (96%).

2-((5-(4-(1H-Benzimidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-phenylethan-1-one (4a)

Yields: 85%. Mp 255–257 °C. FTIR (ATR, cm^{-1}): 3273 (N–H), 2958 (C–H), 1680 (C=O), 698, 742, 850. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.23$ (2 H, s, $–CH_2–$), 7.24–7.26 (2H, m, Benzimidazole CH), 7.59–7.77 (5H, m, Benzimidazole CH, monosubstitutebenzene), 8.09–8.13 (4H, m, monosubstitutebenzene, 1,4-disubstitutebenzene), 8.37 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 13.15 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 41.3$, 110.8, 111.5, 117.5, 121.0, 122.7, 123.5, 125.8, 127.5, 128.8, 129.1, 129.8, 131.1, 134.5, 141.0, 142.3, 160.4, 165.4, 192.2. HRMS (m/z): $[M + H]^+$calcd for $C_{23}H_{16}N_4O_2S$: 413.1067; found: 413.1057.

2-((5-(4-(1H-Benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-(4-methylphenyl)ethan-1-one (4b)

Yields: 80%. Mp 250–252 °C. FTIR (ATR, cm^{-1}): 3367 (N–H), 1662 (C=O), 846. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.43$ (3 H, s, $-CH_3$), 5.20 (2 H, s, $-CH_2-$), 7.26 (2H, y, benzimidazole CH), 7.42 (2 H, d, $J = 8.0$ Hz, 1,4-disubstitutebenzene), 7.64 (2 H, y, benzimidazole CH), 8.00 (2 H, d, $J = 8.2$ Hz, 1,4-disubstitutebenzene), 8.14 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.38 (2 H, d, $J =$ 8.4 Hz, 1,4-disubstitutebenzene), 13.15 (1 H, s, $-$NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.7$, 41.0, 111.9, 166.3, 118.2, 120.8, 122.6, 124.1, 125.7, 127.5, 128.8, 129.1, 129.7, 133.0, 133.6, 145.1, 150.4, 164.2, 165.3, 192.7. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{18}N_4O_2S$: 427.1223; found: 427.1219.

4-(2-((5-(4-(1H-Benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl) thio) acetyl)benzonitrile (4c)

Yields: 81%. Mp 291–293 °C. FTIR (ATR, cm^{-1}): 3280 (N–H), 2958 (C–H), 1681 (C=O), 850. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.25$ (2H, s, $–CH_2–$), 7.26 (2H, dd, $J_1 = 8.4$ Hz, $J_2 = 12.8$ Hz, benzimidazole CH), 7.58 (1H, d, $J = 7.7$ Hz, benzimidazole CH), 7.72 (1H, d, J = 7.7 Hz, benzimidazole CH), 8.11 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.14 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.25 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.38 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 13.15 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 41.1$, 112.1, 116.3, 119.7, 121.1, 123.6, 124.4, 127.5, 127.7, 129.6, 132.9, 133.4, 138.8, 139.1, 144.3, 150.4, 161.2, 163.9, 165.4, 192.8. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{15}N_5O_2S$ 438.1019; found: 438.1010.

2-((5-(4-(1H-Benzimidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-yl) thio) -1-(4-nitrophenyl) ethan-1-one (4d)

Yields: 83%. Mp 256–258 °C. FTIR (ATR, cm^{-1}): 3383 (N–H), 2995 (C–H), 1683 (C=O), 852. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.29$ (2 H, s, $–CH_2–$), 7.22–7.29 (2 H, m, benzimidazole CH), 7.58 (1H, d, $J =$ 7.7 Hz, benzimidazole CH), 7.72 (1H, d, $J = 7.5$ Hz, benzimidazole CH), 8.14 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.33 (2H, d, $J = 8.7$ Hz, 1,4-disubstitutebenzene), 8.38 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.42 (2H, d, $J = 8.7$ Hz, 1,4-disubstitutebenzene), 13.14 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 41.3$, 112.1, 119.7, 120.1, 122.5, 124.5, 126.2, 127.5, 128.4, 130.4, 131.3, 133.6, 140.2, 144.3, 146.8, 150.4, 163.8, 165.4, 192.6. HRMS (m/z): $[M + H]^+$ calcd for $C_{23}H_{15}N_5O_4S$: 458.0918; found: 458.0901.

2-((5-(4-(1H-Benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-(4-chlorophenyl) ethan-1-one (4e)

Yields: 82%. Mp 256–258 °C. FTIR (ATR, cm^{-1}): 3248 (N–H), 2920 (C–H), 1695 (C=O), 846. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.22$ (2H, s, $–CH_2–$), 7.26 (2H, dd, $J_1 = 8.4$ Hz, $J_2 = 13.0$ Hz, benzimidazole CH), 7.58 (1H, d, $J = 7.7$ Hz, benzimidazole CH), 7.68–7.72 (3H, m, benzimidazole CH, 1,4-disubstitutebenzene), 8.11–8.14 (4H, m, 1,4-disubstitutebenzene), 8.38 (2H, d, J = 8.5 Hz, 1,4-disubstitutebenzene), 13.15 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 40.9$, 112.1, 119.7, 122.5, 123.1, 124.1, 126.9, 127.5, 127.7, 129.5, 130.9, 131.2, 133.6, 139.5, 144.3, 150.4, 163.9, 165.3, 192.3. HRMS (m/z): $[M + H]^+$ calcd for $C_{23}H_{15}N_4O_2SCl$: 447.0677; found: 447.0657.

2-((5-(4-(1H-Benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-(4-bromophenyl) ethan-1-one (4f)

Yields: 79%. Mp 282–284 °C. FTIR (ATR, cm^{-1}): 3454 (N–H), 2918 (C–H), 1674 (C=O), 844. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.21$ (2H, s, $–CH_2–$), 7.26 (2H, y, benzimidazole CH), 7.58 (1H, y, benzimidazole CH), 7.71 (1H, y, benzimidazole CH), 7.84 (2H, d, $J =$ 8.5 Hz, 1,4-disubstitutebenzene), 8.03 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.14 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.38 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 13.15 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 40.9$, 113.4, 121.1, 122.4, 124.1, 127.5, 127.7, 128.7, 130.9, 131.0, 132.5, 133.2, 134.6, 139.5, 140.9, 150.4, 163.9, 165.3, 192.5. HRMS (m/z): $[M + H]^+$ calcd for $C_{23}H_{15}N_4O_2SBr$ 491.0172; found: 491.0159.

2-((5- (4- (1H-Benzimidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-(2,4-difluorophenyl) ethan-1-one (4g)

Yields: 77%. Mp 262–264 °C. FTIR (ATR, cm^{-1}): 3325 (N–H), 1614 (C=O), 844. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.09$ (2H, s, –CH_2–), 7.22–7.25 (2H, m, benzimidazole CH), 7.52–7.65 (5H, m, benzimidazole CH, 1,2,4-trisubstitutebenzene), 8.13 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 8.40 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 13.27 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 44.07$, 105.7 (t, $J = 26.80$ Hz), 106.1, 111.3, 113.0 (dd, $J = 23.98$–3.30 Hz), 119.8, 121.1, 123.4, 124.4, 128.2, 128.9, 130.1, 132.4, 133.3 (dd, $J =$ 10.96–3.80 Hz), 133.7, 137.5, 140.2, 142.4, 150.4, 163.9 (dd, $J = 235.1$–11.7 Hz), 165.3 (dd, $J = 255.0$–12.8 Hz), 189.7 (d, $J = 3.76$). HRMS (m/z): $[M + H]^+$ calcd for $C_{23}H_{14}N_4O_2F_2S$: 449.0878; found: 449.0867.

2-((5- (4-(1H-Benzimidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-(2,4-dichlorophenyl) ethan-1-one (4h)

Yields: 81%. Mp 282–284 °C. FTIR (ATR, cm^{-1}): 3325 (N–H), 2974 (C–H), 1670 (C=O), 848. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.07$ (2 H, s, –CH_2–), 7.25–7.27 (2H, m, benzimidazole CH), 7.66–7.68 (3 H, m, benzimidazole CH, 1,2,4-trisubstitutebenzene), 7.83 (1H, d, $J = 2.0$ Hz, 1,2,4-trisubstitutebenzene), 7.97 (1H, d, $J =$ 8.4 Hz, 1,2,4-trisubstitutebenzene), 8.14 (2 H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.38 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 13.16 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 42.9$, 114.4, 118.7, 121.1, 124.1, 127.5, 127.7, 128.2, 128.8, 129.2, 130.8, 132.0, 132.2, 132.3, 133.1, 135.3, 137.6, 150.4, 163.7, 165.4, 193.9. HRMS (m/z): $[M + H]^+$ calcd for $C_{23}H_{14}N_4O_2SCl_2$: 481.0287; found: 481.0274.

2-((5-(4-(1H-Benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-(3,4-dihydroxyphenyl) ethane-1-one (4i)

Yields: 78%. Mp 263–265 °C. FTIR (ATR, cm^{-1}): 3317 (N–H), 2974 (C–H), 1653 (C=O), 848. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.05$ (2H, s, –CH_2–), 6.73 (1H, d, $J = 8.4$ Hz, 1,3,4-trisubstitutebenzene), 7.24–7.26 (2H, m, benzimidazole CH), 7.35 (1H, d, $J = 2.2$ Hz, 1,3,4-trisubstitutebenzene), 7.48 (1H, dd, $J_1 = 2.1$ Hz, $J_2 =$ 8.4 Hz, 1,3,4-trisubstitutebenzene), 7.65 (2H, y, benzimidazole CH), 8.14 (2H, d, $J = 8.6$ Hz, 1,4-disubstitutebenzene), 8.38 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta =$ 40.9, 113.9, 115.3, 122.4, 122.6, 123.0, 123.1, 124.6, 127.5, 128.2, 129.0, 130.2, 133.5, 138.7, 140.6, 146.7, 150.5, 152.2, 164.5, 165.2, 190.1. HRMS (m/z): $[M + H]^+$ calcd for $C_{23}H_{16}N_4O_4S$: 445.0965; found: 445.0943.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl) thio)-1-phenylethan-1-one (4j)

Yields: 85%. Mp 257–259 °C. FTIR (ATR, cm^{-1}): 3336 (N–H), 2914 (C–H), 1681 (C=O), 852. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.45$ (3H, s, –CH_3), 5.23 (2H, s, –CH_2–), 7.08 (1H, y, benzimidazole CH), 7.37–7.48 (2H, m, benzimidazole CH), 7.62 (2H, t, $J = 7.7$ Hz, monosubstitutebenzene), 7.75 (1H, t, $J = 7.5$ Hz, monosubstitutebenzene), 8.11 (4H, d, $J =$ 8.4 Hz, monosubstitüuebenzen, 1,4-disubstitutebenzene), 8.34 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 12.98 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.8$, 41.0, 111.5, 116.8, 118.4, 120.4, 122.4, 123.9, 125.9, 127.4, 128.8, 129.1, 133.7, 134.5, 135.5, 142.7, 150.8, 164.1, 165.3, 193.2. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{18}N_4O_2S$: 427.1223; found: 427.1216.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(4-methylphenyl) ethan-1-on (4k)

Yields: 85%. Mp 260–263 °C. FTIR (ATR, cm^{-1}): 3286 (N–H), 2974 (C–H), 1668 (C=O), 850. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.43$–2.45 (6H, m, –CH_3), 5.19 (2H, s, –CH_2–), 7.05–7.09 (1H, m, benzimidazole CH), 7.35–7.59 (4H, m, benzimidazole CH, 1,4-disubstitutebenzene), 7.99 (2H, d, $J = 8.2$ Hz, 1,4-disubstitutebenzene), 8.11 (2H, d, $J =$ 8.5 Hz, 1,4-disubstitutebenzene), 8.34 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 12.97 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.7$, 21.9, 41.1, 111.6, 119.2, 123.9, 125.1, 127.4, 129.1, 129.9, 131.5, 132.1, 133.0, 133.7, 135.8, 142.5, 145.1, 144.7, 164.1, 165.3, 192.6. HRMS (m/z): $[M + H]^+$ calcd for $C_{25}H_{20}N_4O_2S$: 441.1380; found: 441.1366.

4-(2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)acetyl)benzonitrile (4l)

Yields: 82%. Mp 293–295 °C. FTIR (ATR, cm^{-1}): 3344 (N–H), 2974 (C–H), 1681 (C=O), 848. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.45$ (3 H, s, –CH_3), 5.27 (2H, s, –CH_2–), 7.07–7.09 (1H, m, benzimidazole CH), 7.48 (2H, y, benzimidazole CH), 8.10–8.36 (8H, m, 1,4-disubstitutebenzene), 12.99 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta =$ 21.8, 41.1, 116.3, 118.5, 119.3, 120.9, 121.7, 123.9, 124.1, 126.0, 127.4, 127.5, 129.0, 130.0, 133.4, 138.8, 139.6, 142.4, 163.8, 165.4, 192.8. HRMS (m/z): $[M + H]^+$ calcd for $C_{25}H_{17}N_5O_2S$: 452.1176; found: 452.1158.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(4-nitrophenyl) ethan-1 -on (4m)

Yields: 84%. Mp 276–278 °C. FTIR (ATR, cm^{-1}): 3332 (N–H), 2974 (C–H), 1653 (C=O), 850. ^{1}H-NMR

(300 MHz, DMSO-d_6): $\delta = 2.45$ (3 H, s, $-CH_3$), 5.29 (2H, s, $-CH_2-$), 7.07–7.08 (1H, m, benzimidazole CH), 7.39–7.55 (2H, m, benzimidazole CH), 8.13 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.32–8.36 (4H, m, 1,4-disubstitutebenzene), 8.42 (2H, d, $J = 8.9$ Hz, 1,4-disubstitutebenzene), 12.99 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.8$, 41.3, 112.5, 119.8, 120.1, 123.9, 124.5, 125.9, 127.5, 129.8, 130.7, 131.2, 133.4, 140.2, 142.2, 146.3, 150.8, 163.8, 165.4, 192.6. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{17}N_5O_4S$: 472.1074; found: 472.1060.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(4-chlorophenyl) ethan-1 -on (4n)

Yields: 84%. Mp 292–294 °C. FTIR (ATR, cm^{-1}): 3340 (N–H), 2974 (C–H), 1674 (C=O), 844. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.42$ (3H, s, $-CH_3$), 5.18 (2H, s, $-CH_2-$), 7.03–7.06 (1H, m, benzimidazole CH), 7.33–7.39 (2H, m, benzimidazole CH), 7.66 (2H, d, $J =$ 7.7 Hz, 1,4-disubstitutebenzene), 8.08 (4H, d, $J = 7.6$ Hz, 1,4-disubstitutebenzene), 8.32 (2H, d, $J = 7.8$ Hz, 1,4-disubstitutebenzene), 12.96 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.7$, 40.8, 116.9, 119.8, 120.7, 122.5, 124.1, 127.5, 127.6, 129.3, 129.52, 130.91, 134.2, 135.9, 139.3, 147.1, 150.3, 160.9, 163.8, 192.5. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{17}N_4O_2SCl$: 461.0834; found: 461.0821.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(4-bromophenyl) ethan-1 -on (4o)

Yields: 78%. Mp 283–285 °C. FTIR (ATR, cm^{-1}): 3462 (N–H), 2918 (C–H), 1674 (C=O), 848. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.45$ (3H, s, $-CH_3$), 5.21 (2H, s, $-CH_2-$), 7.08 (1H, y, benzimidazole CH), 7.36–7.59 (2H, m, benzimidazole CH), 7.84 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.03 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.12 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.35 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 12.98 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.8$, 41.0, 111.7, 119.3, 123.9, 124.2, 124.5, 127.4, 127.5, 128.7, 129.8, 131.2, 132.5, 134.7, 139.6, 140.1, 142.5, 163.9, 165.4, 192.5. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{17}N_4O_2SBr$: 505.0328; found: 505.0316.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(2,4-difluorophenyl) ethane-1-one (4p)

Yields: 71 %. Mp 266-268 °C. FTIR (ATR, cm^{-1}): 3456 (N–H), 3066 (C–H), 1662 (C=O), 821. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 3.35$ (3H, s, $-CH_3$), 5.06 (2H, s, $-CH_2-$), 7.04–7.07 (1H, m, benzimidazole CH), 7.28–7.57 (4H, m, benzimidazole CH, 1,2,4-trisubstitutebenzene), 8.02–8.07 (1H, m, 1,2,4-trisubstitutebenzene), 8.10 (2H, d, $J = 8.6$ Hz, 1,4-disubstitutebenzene), 8.34 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 12.98 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.7$, 41.4, 105.8 (t, $J = 26.30$ Hz), 106.5, 112.3, 113.2 (dd, $J = 23.90$–3.30 Hz), 119.7, 122.1, 123.7, 124.4, 128.7, 128.9, 130.3, 132.6, 133.3 (dd, $J = 10.96$ Hz-3.80 Hz), 133.9, 138.5, 140.1, 142.4, 150.9, 163.9 (dd, $J =$ 235.0–11.7 Hz), 165.2 (dd, $J = 255.0$–12.8 Hz), 190.7 (d, $J = 3.76$). HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{16}N_4O_2F_2S$: 463.1035; found: 463.1017.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(2,4-dichlorophenyl) ethane-1-one (4r)

Yields: 80%. Mp 280–283 °C. FTIR (ATR, cm^{-1}): 2924 (C–H), 1662 (C=O), 823. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.45$ (3H, s, $-CH_3$), 5.06 (2H, s, $-CH_2-$), 7.03–7.10 (1H, m, benzimidazole CH), 7.35–7.56 (2H, m, benzimidazole CH), 7.66 (1H, dd, $J_1 = 2.0$ Hz, $J_2 = 8.4$ Hz, 1,2,4-trisubstitutebenzene), 7.81 (1H, d, $J = 2.0$ Hz, 1,2,4-trisubstitutebenzene), 7.97 (1H, d, $J = 8.4$ Hz, 1,2,4-trisubstitutebenzene), 8.11 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.35 (2H, d, $J = 8.4$ Hz, 1,4-disubstitutebenzene), 12.97 (1H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta = 21.7$, 41.2, 112.3, 118.3, 119.3, 121.6, 123.5, 125.4, 127.5, 128.2, 129.7, 130.9, 131.2, 132.3, 133.4, 134.1, 138.4, 145.2, 151.7, 162.9, 164.4, 192.2. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{16}N_4O_2Cl_2S$: 495.0444; found: 495.0421.

2-((5-(4-(6-Methyl-1H-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3,4-dihydroxyphenyl) ethane-1-one (4s)

Yields: 85%. Mp 260–262 °C. FTIR (ATR, cm^{-1}): 3334 (N–H), 2972 (C–H), 1651 (C=O), 846. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.43$ (3H, s, $-CH_3$), 5.07 (2H, s, $-CH_2-$), 6.86 (1H, d, $J = 8.2$ Hz, 1,3,4-trisubstitutebenzene), 7.05 (1H, d, $J = 8.3$ Hz, benzimidazole CH), 7.41–7.52 (4H, m, benzimidazole CH, 1,3,4-trisubstitutebenzene), 8.09 (2H, d, $J = 8.5$ Hz, 1,4-disubstitutebenzene), 8.33 (2H, d, $J = 8.3$ Hz, 1,4-disubstitutebenzene). ^{13}C-NMR (75 MHz, DMSO-d_6): $\delta =$ 21.7, 41.0, 112.8, 116.3, 121.8, 123.5, 123.9 124.1, 127.4, 128.6, 129.6, 130.2, 131.2, 133.7, 138.9, 140.5, 146.7, 150.1, 152.4, 164.4, 165.2, 192.2. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{18}N_4O_4S$: 459.1122; found: 459.1101.

Cytotoxicity test

The anticancer activity of compounds **4a–4s** was screened according to the MTT assay. The MTT assay was performed as previously described (Özkay et al. 2016). Cisplatin was used as a reference drug A549 and HepG2 cell lines were used in the MTT assay. In addition to the activity of anticancer drug candidates, it is important that they do not show toxic effects on healthy cell line. For this purpose, the cytotoxic properties of the most active compounds (**4a**, **4c**, and **4f**) on A549 (human lung adenocarcinoma) and HepG2 (human liver cancer cell line) cell line were evaluated by MTT method using NIH3T3 (mouse embryonic fibroblast cells) cell line.

Flow cytometric analysis

Death pathway of the carcinogenic cell lines was detected by Annexin V-FITC Apoptosis Detection Kit (BD, Pharmingen) as reported previously (Acar Cevik et al. 2018). Cisplatin and compounds **4a**, **4c**, and **4f** that possess the highest cytotoxic activity were used at their $IC_{50/2}$, IC_{50}, and $2xIC_{50}$ concentrations. FCSExpress software was used to display the percent of normal and apoptotic cells at different stages. In the diagrams, Q1, Q2, Q3, and Q4 demonstrates the necrotic cells (positive for PI and negative for annexin/FITC), late apoptotic or necrotic cells (positive for annexin and PI), live cells (negative for annexin and PI), and apoptotic cells (negative for PI and positive for annexin), respectively. The experiments were carried out in triplicates.

DNA topoisomerase I activity

In this study, the topoisomerase I assay kit (TG1018-2; TopoGen) was used to determine if synthesized compounds showed topoisomerase I inhibition. The topoisomerase I inhibition activities of final compounds were measured as relaxation of supercoiled plasmid DNA using agarose gel electrophoresis and camptothecin was used as a positive control. The assay was carried out in a final volume of 20 µL reaction volume containing 2 µL of 10xTGS Buffer, 6 µL water, 2 µL supercoiled plasmid DNA, 2 µL test compound, 2 µL of Top1, 2 uL 10% SDS, 2 µL proteinase K, 2 µL DNA loading dye. After incubation of reaction mixtures at 37 °C for 30 min, electrophoresis was done on a 1% agarose gel at a potential 50 V for 75 min using 1xTAE buffer.

Results and discussion

Chemistry

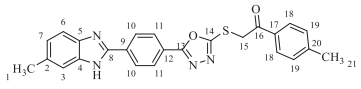
Compounds (**4a–4s**) designed to study biological activity were obtained from four reaction steps. In the first step, benzimidazole derivatives (**1a**, **1b**) were obtained by reaction of phenylenediamine derivatives in dimethylformamide with methyl 4-formyl benzoate compounds in the presence of microwave radiation. In the second step, hydrazine hydrate was added to the solution of benzimidazole compounds (**1a**, **1b**) in ethanol to give 4-(5(6)-substituted-1*H*-benzimidazol-2-yl)benzoic acid hydrazide (**2a**, **2b**) derivatives. In the third step, in order to obtain 5-(4-(5(6)-substituted-1*H*-benzimidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-thiol derivatives (**3a**, **3b**), (6)-substituted-1*H*-benzimidazol-2-yl)benzoic acid hydrazide derivatives (**2a**, **2b**) were reacted with carbon disulfide. In the last step, thiol derivatives (**3a**, **3b**) were reacted with various phenacyl bromide derivatives to give the resulting compounds (**4a**–**4s**). The synthesis scheme for obtaining the target compounds was given in Scheme 1.

The structures of the compounds (**4a–4s**) were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy. In the IR spectrum, C=O bands were observed between 1614 cm^{-1} and 1695. N–H bands of benzimidazole structure were observed between 3248 and 3462 cm^{-1}. In addition for structure elucidations using routine spectroscopic methods, 2D NMR studies including HMBC and HSQC were performed for compound **4k** (see Supplementary Materials).

Cytotoxicity test

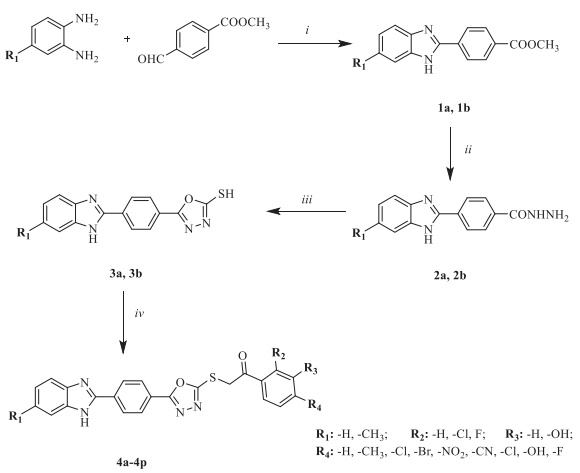
The anticancer activity potential of the synthesized compounds (**4a–4s**) against A549 and HepG2 cell lines at various concentrations (1, 0.316, 0.1, 0.0316, 0.01, 0.00316, 0.001, 0.000316 mM) was evaluated. Furthermore, the cytotoxic activities of these compounds were evaluated against healthy NIH3T3 cells in order to see their selectivity against cancer cells. IC_{50} values for the cell lines of the compounds were presented in Table 1. Compounds **4a** and **4l** were found to have the highest activity against the A549 cell line. The compound **4a** showed activity against the A459 cell line with an IC_{50} value (0.013 mM) lower than cisplatin (0.06 mM). Compounds **4a**, **4c**, **4f**, **4h**, and **4r** were found to have the highest activity against the HepG2 cell line. Compounds **4a**, **4c**, and **4f** showed lower IC_{50} value than cisplatin against HepG2 cell line. Besides, compounds **4h** and **4r** showed activity against HepG2 cell line with the same IC_{50} value (0.06 mM) as cisplatin.

One of the important criteria for being an anticancer agent candidate is that they have as little or no side effects on healthy cells. Therefore, the cytotoxicity of the compounds **4a–4s** against the NIH3T3 cell line was evaluated. Compounds **4a** and **4l** displayed selectivity towards carcinogenic A549 cell line, when compared with healthy NIH3T3 cells. Furthermore, the selectivity of compound **4l** (SI:30) against A549 cells was three times higher than that of compound **4a** (SI:10). The IC_{50} values of the compounds



Compound **4k**

	H	C
1	2,44	21,9
2	-	133,0
3	7,34	111,7
4	-	135,8
5	-	142,5
6	7,06	123,9
7	7,56	119,5
8	-	150,3
9	-	125,1
10	8,09	127,4
11	8,33	127,5
12	-	133,7
13	-	165,3
14	-	164,1
15	5,17	41,1
16	-	192,6
17	-	145,1
18	7,97	129,9
19	7,40	129,1
20	-	131,5
21	2,41	21,7
22	12,96	-



1a, 1b **2a, 2b** **3a, 3b** **4a-4p**

R_1: -H, -CH_3; **R_2**: -H, -Cl, F; **R_3**: -H, -OH;
R_4: -H, -CH_3, -Cl, -Br, -NO_2, -CN, -Cl, -OH, -F

Compound	R_1	R_2	R_3	R_4
4a	H	H	H	H
4b	H	H	H	CH_3
4c	H	H	H	CN
4d	H	H	H	NO_2
4e	H	H	H	Cl
4f	H	H	H	Br
4g	H	F	H	F
4h	H	Cl	H	Cl
4i	H	H	OH	OH
4j	CH_3	H	H	H
4k	CH_3	H	H	CH_3
4l	CH_3	H	H	CN
4m	CH_3	H	H	NO_2
4n	CH_3	H	H	Cl
4o	CH_3	H	H	Br
4p	CH_3	F	H	F
4r	CH_3	Cl	H	Cl
4s	CH_3	H	OH	OH

Scheme 1 The synthetic protocol of the compounds. Reagents and conditions; i: $Na_2S_2O_5$, DMF, Mwı, 10 min, ii: NH_2NH_2 x H_2O, EtOH, Mwı, 10 min, iii: CS_2/NaOH, EtOH, reflux, 8 h, iv: appropriate phenacyl bromides, K_2CO_3, acetone, rt, 8 h

Table 1 IC_{50} (μM) values of compounds **(4a–4s)**

Bileşik	A549	HepG2	NIH3T3
4a	**13 ± 1**	**3 ± 0.7**	**130 ± 8**
4b	310 ± 20	170 ± 10	>1000
4c	370 ± 20	**50 ± 1**	**550 ± 20**
4d	1000 ± 170	80 ± 2	>1000
4e	130 ± 10	40 ± 5	130 ± 20
4f	260 ± 60	**40 ± 8**	**660 ± 80**
4g	30 ± 8	40 ± 7	40 ± 5
4h	100 ± 20	**60 ± 7**	**560 ± 70**
4i	>1000	>1000	>1000
4j	90 ± 8	50 ± 3	300 ± 70
4k	700 ± 9	80 ± 9	>1000
4l	**60 ± 12**	80 ± 1	>1000
4m	290 ± 40	20 ± 6	70 ± 10
4n	160 ± 40	60 ± 10	250 ± 60
4o	450 ± 30	470 ± 10	>1000
4p	110 ± 32	110 ± 20	110 ± 10
4r	300 ± 181	**60 ± 3**	**450 ± 50**
4s	>1000	>1000	>1000
Cisplatin	**60 ± 9**	**60 ± 4**	>1000

The important values obtained according to the result of activity are shown in bold

Table 2 SI values of compounds **4a**, **4c**, **4f**, **4h**, and **4r**

Comp.	SI (HepG2)
4a	43,33
4c	11
4f	16,5
4h	9,33
4r	7,5

4a, **4c**, **4f**, **4h**, and **4s** against the HepG2 cell line were lower than the IC_{50} values against the NIH3T3 cell line. Further, the selectivity of compound **4a** to HepG2 cells was higher than for all compounds.

The selectivity index (SI) values were calculated for the effectiveness of selected compounds against HepG2. The compounds with SI value >3 is considered to exhibit selective inhibition towards cancer cells (Haider et al. 2019). Compounds **4a**, **4c**, **4f**, **4h**, and **4r** exhibited good selectivity against HepG2 cell lines since their SI values were more than 3 (Table 2). The SI suggests that compounds 4b, 4d, and 4e are effective and selective against HeLa cell line.

As seen in the Table 1, HepG2 cell line was more susceptible to compounds **4a**, **4c**, and **4f** than A549 cell line. Therefore, HepG2 cell line was used in further anticancer

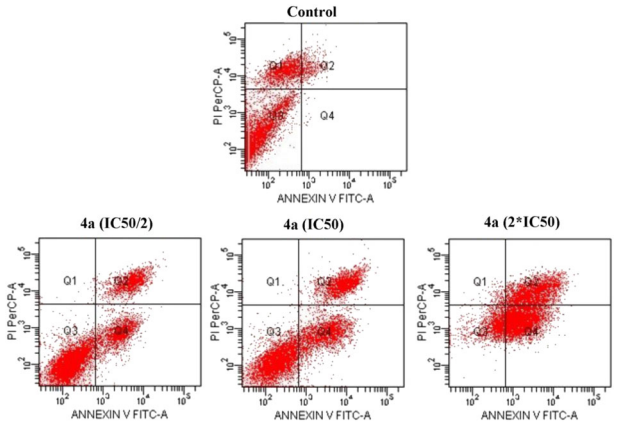


Fig. 1 Flow cytometric analysis diagram of compound **4a** for HepG2 cell line

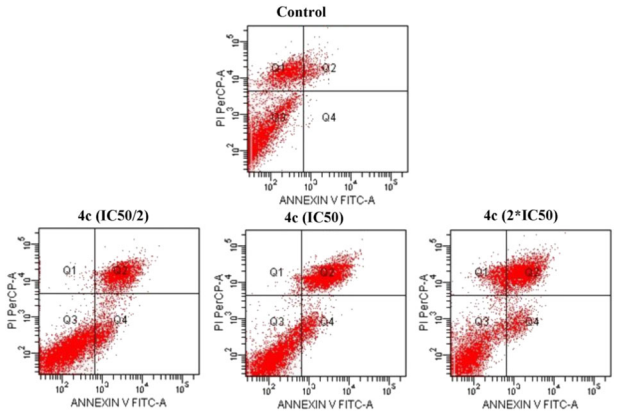


Fig. 2 Flow cytometric analysis diagram of compound **4c** for HepG2 cell line

activity screening tests flow cytometric analysis assay and DNA topoisomerase inhibition assay.

Flow cytometric analysis

Flow cytometry studies were performed for compounds 4a, 4c, 4f, and cisplatin on HepG2 cells. The aim of this study is to determine whether cellular death is induced by apoptosis or necrosis. Flow cytometric analysis diagrams of compounds **4a, 4c, 4f**, and cisplatin at $IC_{50}/2$, IC_{50}, and $2xIC_{50}$ concentrations on HepG2 cells are presented in Figs. 1–4. It is determineted that the compounds 4a and 4r showed the highest level of apoptotic cells than cisplatin at the IC_{50}. These results estimated that after 24 h treatment concentration compounds **4a** and **4r** provoked the apoptotic induction in HepG2 cell line. The percent values of apoptotic cells at concentrations of $2 \times IC_{50}$, IC_{50}, and $IC_{50/2}$ for compounds **4a**, **4c**, and **4f** were given in Table 3.

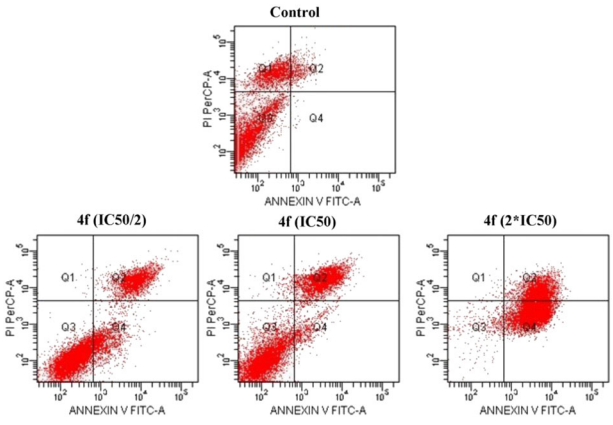


Fig. 3 Flow cytometric analysis diagram of compound **4f** for HepG2 cell line

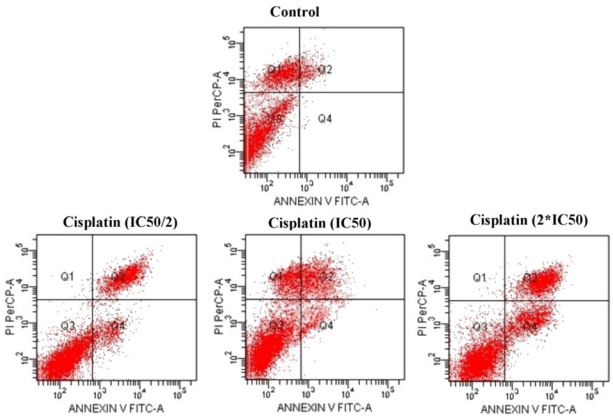


Fig. 4 Flow cytometric analysis diagram of cisplatin for HepG2 cell line

Table 3 Percentage of apoptotic cells at $2 \times IC_{50}$, IC_{50}, and $IC_{50/2}$ concentration for compounds **4a**, **4c**, and **4f**

Compound	Q1	Q2	Q3	Q4
4a				
$IC_{50}/2$	0.3	16.4	64.4	18.9
IC_{50}	0.2	23.1	50.4	26.3
$2 \times IC_{50}$	1.8	29.4	17.8	51.0
4c				
$IC_{50}/2$	15.6	3.8	80.4	0.2
IC_{50}	11.2	27.7	53.8	7.3
$2 \times IC_{50}$	9.7	36.0	47.3	7.0
4f				
$IC_{50}/2$	0.2	22.9	62.5	14.4
IC_{50}	1.3	32.5	50.5	15.7
$2 \times IC_{50}$	0.3	27.3	40.1	68.3
Cisplatin				
$IC_{50}/2$	0.3	18.4	73.0	8.3
IC_{50}	3.2	20.4	66.0	15.3
$2 \times IC_{50}$	0.3	24.1	58.9	16.8

DNA topoisomerase I inhibition

The DNA topo I inhibition test was performed according to the Topoisomerase I Drug Screening Kit procedure (Topogen). This kit is used to determine whether compounds that inhibit Topo I activity act as catalytic inhibitors or as topoisomerase poisons. The tested compounds were tested at one concentration of 50 mM. Camptothecin was used as a control topoisomerase I poison. Compound **4a**, **4c**, and **4f** did not inhibit topoisomerase I, while camptothecin inhibited topoisomerase I enzyme (see Supplementary Materials).

Conclusion

In this study, we synthesized new 2-[(5-(4-(5(6)-substituted-1*H*-benzimidazol-2-yl)phenyl)-1,3,4-oxadiazol-2-yl)thio]-1-(4-substitutedphenyl)ethan-1-ones (**4a–4s**) and evaluated their anticancer potency against carcinogenic A549 and HepG2 cell lines. In addition, the cytotoxicity of the synthesized compounds against the healthy NIH / 3T3 cell line was evaluated to calculate the SIs. It was determined that compounds **4a, 4c**, and **4f** showed significant activity against HepG2 cell line. But, these compounds did not inhibit DNA topo I.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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